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14. ABSTRACT

Protein kinase C epsilon (PKCε), a member of the PKC family of phorbol ester/diacylglycerol receptors, is up-regulated in many human cancers, including prostate cancer. We recently demonstrated that PKCε is an essential mediator of NF-κB activation in prostate cancer (Garg et al., JBC, 287, 37570–37582, 2012). In this research, we wish to determine if PKCε regulates TNFα-signaling to mediate its effect on NF-κB activation. Using a specific PKCε antagonist, we demonstrated that PKCε plays essential role in the TNFα-induced phosphorylation of TNF receptor in prostate cancer cells.

We have previously identified that PKCε regulates NF-κB responsive genes in prostate cancer cells, including cyclooxygenase-2 (COX-2) (JBC, 2012). COX-2 has been reported to be up-regulated in metastatic prostate cancer. As PKCε plays an important role in prostate cancer cell survival and cooperates with other oncogenic insults, herein we aim to determine if PKCε regulates COX-2 activation during prostate tumorigenesis. In the previous funding period we have demonstrated that PKCε mediates the activation of COX-2, a well-known NF-κB responsive gene in prostate cancer and that COX-2 mediates PKCε responses in prostate cancer.

In the present report, we present our continued efforts on the in-depth determination of the role of PKCε in COX-2 activation in prostate cancer and also to investigate if COX-2 is a potential mediator of PKCε oncogenesis in prostate cancer, particularly in the context of Pten loss and to determine if COX-2 inhibition could also affect the signaling event in adenocarcinoma formed in the compound PKCε;Pten mice. Our results showed that PKCε cooperates with Pten loss to activate COX-2 and some other key relevant signaling molecules (Akt, S6, mTOR, Stat3, NF-kB) in mouse model of prostate cancer. COX-2 inhibition by dietary rofecoxib was found to impair the activation of signaling pathways in adenocarcinomas formed as a consequence of PKCε overexpression and Pten loss in mice. Additionally, PKCε overexpression was found to cooperate with Pten loss to enhance the activation of signaling events (Erk, Akt and mTOR) in response to growth factors relevant in prostate cancer, in murine cellular model. Furthermore, global gene expression by microarray revealed significant changes in gene related to EMT, adhesion, metabolism, and invasiveness following PKCε overexpression, Pten loss or both. More importantly model of PKCε overexpression and Pten loss (CaP8-PKCε cells which also showed enhanced COX-2 activation) displays a similar pattern of expression than that observed in prostate cancer metastasis in human patients. Both canonical and non-canonical NF-κB pathways are involved in the COX-2 activation mediated by PKCε overexpression and Pten loss.

15. SUBJECT TERMS

PKC Epsilon, Prostate Cancer, COX-2, Pten

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INTRODUCTION

Prostate cancer is the second leading cause of cancer-related deaths among men in the US. The American Cancer Society estimates 230,000 new cases and nearly 30,000 deaths for 2015. Despite recent advances in diagnosis and treatment, many patients who underwent treatment still experience recurrent, androgen-independent prostate cancer, for which there are limited treatment options. Most common alterations in prostate tumors include the functional inactivation/deletion of tumor suppressors (e.g. Pten, p53, or Nkx3.1), deregulation of growth factor signaling (e.g. IGF-1R and ErbB receptors) and their effectors (e.g. PI3K), and genomic rearrangements (e.g. TMPRSS2-ERG)[1-7]. Critical links between inflammatory pathways, such as NF-κB, and malignant progression have also been established in prostate cancer[8-13]. Efforts to develop therapeutic approaches and chemopreventive strategies (both dietary and pharmacological) to mitigate the prostate cancer burden have been only partially successful, largely due to the incomplete picture of the mechanisms orchestrating prostate cancer development and progression.

Studies have recognized protein kinase C (PKC) isozymes as eminent players of cancer progression[14-19]. Based on biochemical and structural differences, these Ser-Thr kinases have been classified into calcium-dependent "classical" cPKCs (α/β/γ), calcium-independent "novel" nPKCs (δ/ε/η/θ), and atypical PKCs (ζ and λ/ι)[14, 19]. Individual PKCs have dissimilar roles in controlling cancer signaling pathways such as Ras/Erk, PI3K/Akt, and NF-κB, thereby reflecting their distinctive involvement in cancer progression. Emerging information established PKCε as an oncogenic kinase and cancer biomarker[14, 19-27]. Early studies revealed that overexpression of PKCε in fibroblasts or epithelial cells confer growth advantage or can lead to malignant transformation[14, 20, 25]. Accordingly, PKCε is a pro-survival (anti-apoptotic) kinase, and it has been widely implicated in motility/invasion/metastasis, including in the secretion of metalloproteases[22-24, 28-33]. A very interesting fact is that up-regulation of PKCε occurs in human cancer, as extensively demonstrated in prostate, breast, lung, ovarian, and head and neck cancer [14, 19, 22-27, 33]. PKCε is essentially undetectable in normal prostate epithelium or benign prostatic epithelium, however it is highly expressed in the majority of prostate tumors and in recurrent disease[17-18]. PKCε genetic ablation impairs tumor formation and metastasis in the TRAMP mouse model of prostate cancer[24]. Central roles for PKCε have also been established in the progression of other cancers, such as melanoma, breast and lung cancer[19, 23, 26, 32-33]. Regardless, it is not yet clear if PKCε overexpression is causally related to the initiation and progression of the disease. Likewise, the mechanistic basis of the PKCε mitogenic, survival and oncogenic activity, as well as the PKCε downstream effectors, remain to be fully elucidated.

We have generated transgenic models to target PKC isozymes to the mouse prostate under the control of the androgen-responsive probasin (PB) promoter[29]. Interestingly, only PB-PKCε mice developed dysplastic changes characteristic of prostatic intraepithelial neoplasia (PIN). Strikingly, prostate-specific PKCε transgenic mice when intercrossed with the mice haploinsufficient for Pten, another common genetic alteration in human prostate cancer, resulted in a compound mutant mice (PB-PKCε;Pten+/- mice) that developed fully invasive adenocarcinoma. We recently demonstrated that transgenic overexpression of PKCε in the mouse prostate causes preneoplastic lesions with elevated NF-κB levels and that PKCε is an essential mediator of NF-κB activation in prostate cancer[8].

In the previous DOD funding period, we specifically identified a functional link between the oncogenic kinase PKCε and inducible cyclooxygenase-2 (COX-2), a well-known NF-κB responsive gene.

Cyclooxygenase (COX) converts arachidonic acid to PGE2, PGD2, PGI2, PGF2α and thromboxane A2 (TXA2), collectively the prostanoids[34-36]. Up-regulated expression of COX-2 is an early event during carcinogenesis, and it has been mostly associated with poor prognosis[37-39]. Cell, animal, and clinical studies ascertained key roles for COX-2 in tumor formation and progression across a range of cancers. Notably, COX-2 has been reported to be elevated in multiple solid tumors, including prostate, colorectal, breast, and pancreatic cancer[40-44]. Concordantly, studies reported that COX-2 is overexpressed in primary prostate cancer with metastatic potential and predicts poor patient survival[38-39, 43-45]. Selective COX-2 inhibitors reduce proliferation and induce apoptosis in prostate cancer cells, as well as inhibit the growth of prostate tumors and tumor angiogenesis in nude mice[43, 45-48].

In the last year progress report, we have demonstrated that:

(i) Targeted PKCε overexpression in normal prostate epithelium leads to COX-2 activation.

(ii) To understand in depth the underlying molecular mechanisms of PKCε-mediated phenotype in prostate cancer, we generated mouse cellular models overexpressing PKCε both in Pten (a tumor suppressor gene) + and – background(s) to evaluate effect on COX-2 activation during prostate carcinogenesis.

(iii) PKCε overexpression and Pten loss cooperate for cell proliferation, growth, migration and invasiveness.

(iv) PKCε overexpression and Pten loss cooperate for NF-κB activation.

(v) PKCε overexpression and Pten loss cooperate for enhanced COX-2 activation and PGE2 production.

(vi) COX-2 inhibition specifically impaired the viability of PKCε overexpressing cells.

(vii) COX-2 play a critical role in PKCε mediated survival of murine prostate cells.

(viii) COX-2 inhibition reduced the PGE2 synthesis specifically in PKCε overexpressors.

(ix) COX-2 inhibition decreased the invasiveness of PKCε overexpressors.

(x) COX-2 inhibition inhibited the growth of CaP8-PKCε tumors in athymic nude mice and activates apoptotic pathway.

(xi) A correlation between PKCε levels, NF-κB hyperactivation and COX-2 up-regulation was demonstrated in human prostate cancer specimens.

The main goal of our continued research supported by DOD is to determine if PKCε and Pten mediate COX-2 activation in mice prostate and to determine the levels of relevant signaling molecules and to understand the hidden underlying mechanism of activation.

BODY

1. **Enhanced signaling alterations in lesions from PKCε transgenic mice.** In order to establish a molecular signature of lesions in PB-PKCε and PB-PKCε;Pten+/- mice we carried out immunohistochemistry (IHC) analysis of prostates from 12-month old mice. We found elevated phospho (activated)-Akt staining in PIN lesions, an effect that was more striking in adenocarcinimas from PB-

PKCε;Pten+/- mice. Akt downstream effectors S6 and mTOR were also hyperactivated in PINs and adenocarcinomas (Fig. 1A), suggesting the involvement of the PI3K/Akt axis in the phenotypic changes driven by PKCε overexpression. We also observed elevated levels of Stat3, phospho-Stat3, and nuclear NF-κB (Fig. 1A and B). Similar increase in the protein expression level of these signaling markers were observed by Western blot analysis in the total cell lysate (for Akt, mTOR, Stat3) and in the nuclear lysate for NF-κB (Fig. 1C and D). Strikingly, PKCε cooperates with Pten loss to further enhance the protein expression of these relevant molecular signatures. These findings are highly significant because both Pten loss and PKCε overexpression are common alterations in human prostate cancer.

2. **Enhanced COX-2 activation in lesions from PKCε;Pten transgenic mice.** As evident in Fig. 2 enhanced COX-2 levels were observed in total cell extracts from prostates of PB-PKCε mice and the effect is further enhanced in adenocarcinomas from PB-PKCε-Pten+/-mice.

3. **COX-2 inhibition impairs the activation of signaling pathways in adenocarcinomas formed as a consequence of PKCε overexpression and Pten loss.** In order to determine the relevance of COX-2 in the context of PKCε overexpression, we fed the PB-PKCε-Pten+/-mice with the diet containing COX-2 inhibitor, rofecoxib. For this experiment, PB-PKCε-Pten+/-mice were divided in 2 groups. Group 1 received the control laboratory diet. Group 2 mice were fed with rofecoxib (COX-2 inhibitor) diet. Notably, total cell extracts from prostates of mice fed with rofecoxib diet showed significant reduction in the activation of key signaling pathways including phosphorylation of Akt, its downstream effector mTor and also the phosphorylation of Stat3 as compared to the adenocarcinomas from mice receiving control diet (Fig. 3 A and B).

4. **PKCε overexpression and Pten loss cooperate for Erk, Akt and mTOR activation.** In the last funding period, we have generated murine cellular models of PKCε overexpression and Pten loss recapitulating the scenario observed in our transgenic mice model. The strong cooperativity between PKCε overexpression and loss of the Pten tumor suppressor gene was observed in these cellular models for growth, motility and invasiveness; suggestive of changes in key signaling events. Notably, as can be seen in Fig. 4, a remarkable cooperation for signaling activation was observed upon PKCε overexpression and Pten loss. CaP8-PKCε cells with PKCε overexpression and Pten loss show significant activation of Akt and mTOR in response to PDGF, thus recapitulating results observed in lesions from PB-PKCε;Pten+/- mice. A similar effect was observed for Erk.

5. **Gene expression analysis in murine prostate epithelial cell lines.** As PKCε overexpression and Pten loss have significant influence on the COX-2 activation, a gene that is upregulated in metastatic prostate cancer, we next intended to fully elucidate the global expression changes that might occur in the various molecular signatures as a result of PKCε overexpression and Pten loss. Towards this goal, we conducted a microarray analysis with the four murine prostate epithelial cell line (P8, P8-PKCε, CaP8, CaP8-PKCε) that we generated recapitulating the scenario observed in our transgenic mice model. Microarray analysis of P8-derived cell lines revealed major changes in gene expression by PKCε overexpression, Pten loss, or both (Fig. 5). After statistical testing procedures, we identified in total 573, 898 and 1101 genes respectively in CaP8, P8-PKCε and CaP8-PKCε genotypes that were

significantly dysregulated when compared to P8 parental background. In addition, Venn-diagram analysis shown in Fig. 5A not only reveal the specificity and intersection of transcripts but also clearly describes the distribution of significantly differentially expressed genes (both up- and down-regulated) ensuing comparison amongst different groups with reference to P8. Notably, though 289, 487, 556 genes were upregulated in CaP8, P8-PKCε and CaP8-PKCε categories respectively either singly or in consortium, 263 genes were found to be exclusively up-regulated in CaP8-PKCε group vs P8. Likewise, in spite of an overlap of down-modulated genes in different backgrounds, our analysis identified another set of 263 genes that were uniquely down-regulated in CaP8-PKCε genotype vs P8. Hierarchical cluster analysis further demonstrated that PKCε overexpression in the context of Pten loss (CaP8-PKCε) has significant impact on the gene expression profile compared to P8. Heat map shown in Fig. 5B markedly demarcate up/down-regulated genes in the two genotypes. To identify the most relevant functions/pathways altered by PKCε overexpression and/or Pten loss in prostate epithelial cells, we used Gene Ontology (GO) and Kyoto Enclopedia Genes and Genomes (KEGG) databases (Fig 5C). This analysis uncovered major changes in key functions, including metabolic pathways, angiogenesis, motility, and proliferation and identified an important chemokine CXCL13 playing crucial role in prostate carcinogenesis. We then compared results from our microarray gene set analysis with a publicly available dataset of prostate cancer patients (GSE6919 dataset, 167 patients). Interestingly, this comparison revealed that the model of PKCε overexpression and Pten loss (CaP8-PKCε cells which have high COX-2 activation) displays a similar pattern of expression than that observed in prostate cancer metastasis (Fig. 5D).

6. **Inhibition of both canonical and non-canonical NF-κB pathway decreased the COX-2 activation mediated by PKCε overexpression and Pten loss.** As COX-2 is known to be a NF-κB regulated gene, we dissected if canonical or non-canonical NF-κB pathway is associated with the activation of COX-2 observed as a result of PKCε overexpression and Pten loss. As can be seen in Fig 6A, LPS treatment resulted in significant activation of COX-2 in murine prostate epithelial cells and this effect is more pronounced in CaP8-PKCε cells. To inhibit canonical or non-canonical NF-κB pathway we used RNAi approach. We silenced the expression of NIK, IKKα, IKKβ expression in the CaP8-PKCε cells as well as we used IκBα repressor plasmid to specifically affect the canonical pathway. As can be seen in Fig. 6B silencing the expression of NIK, IKKα, IKKβ significantly reduced the LPS-induced COX-2 mRNA levels. Similar effect was observed in response to the IκBα repressor (Fig. 6C). These results suggest the involvement of both canonical and non-canonical NF-κB pathway in COX-2 induction as a consequence of PKCε overexpression and Pten loss in prostate cells. We confirmed the knockdown mediated by different RNAi used by western blot (Fig. 6D) and the activity of IκBα repressor plasmid by measuring NF-κB luciferase activity.

KEY RESEARCH ACOMPLISHMENTS

(i) We successfully demonstrated that PKCε cooperates with Pten loss to activate key relevant signaling molecules (Akt, S6, mTOR, Stat3, NF-kB) in mouse model of prostate cancer using IHC and western blot approaches.

(ii) We successfully demonstrated that PKCε cooperates with Pten loss to activate COX-2 in mouse model of prostate cancer.

(iii) We successfully demonstrated that COX-2 inhibition impairs the activation of signaling pathways in adenocarcinomas formed as a consequence of PKCε overexpression and Pten loss.

(iv) We successfully demonstrated that in murine cellular model of PKCε overexpression and Pten loss, a striking enhancement in the signaling events (in particular of Erk, Akt and mTOR activation) occurred in response to growth factors relevant in prostate cancer.

(v) Our analysis of global gene expression by microarray revealed significant changes in gene related to EMT, adhesion, metabolism, and invasiveness following PKCε overexpression, Pten loss or both. More importantly model of PKCε overexpression and Pten loss (CaP8-PKCε cells which also showed enhanced COX-2 activation) displays a similar pattern of expression than that observed in prostate cancer metastasis in human patients.

(vi) We successfully demonstrated that inhibiting both canonical and non-canonical NF-κB pathway decreased the COX-2 activation mediated by PKCε overexpression and Pten loss.

CONCLUSION

The main conclusions from the research carried out during the final year of DOD funding are as follows: (i) PKCε mediates the activation of COX-2, a well-known NF-κB responsive gene in prostate cancer. (ii) PKCε cooperates with tumor suppressor Pten leading to the formation of adenocarcinoma in mice which displayed hyperactivation of key signaling pathways and more importantly COX-2 which is highly relevant in metastatic prostate cancer. (iii) our global gene expression profile also confirms the relevance of COX-2 activation together with expression changes in genes associated with important biological pathways that play crucial role in prostate carcinogenesis. These findings suggest a crucial role of PKCε in several important cellular processes relevant to prostate cancer progression, including survival, proliferation, metastasis and invasion. Overall, our study identified COX-2 as a PKCε-regulated gene and also suggests that COX-2 is a potential mediator of PKCε oncogenesis in prostate cancer, particularly in the context of Pten loss. Our findings have been honored at Annual meetings of American Association of Cancer Research 2014 under “Late Breaking Abstract” category and in 2015 with an AACR-Aflac Scholar-in-training award.

REPORTABLE OUTCOMES

1. **Garg, R**., Benedetti, L., Abera, M., Wang, H., Abba, M., and Kazanietz, M.G. Protein kinase C and cancer: what we know and what we don’t. ***Oncogene***, 33: 5225-5237 (2014).

2. **Garg, R.,** Abera, M. B., Lal, P., Blando, J., Benavides, F., Feldman, M. D., Smyth, E. M., and Kazanietz, M.G. COX-2 as a mediator of oncogenic PKCε in prostate cancer. ***Late Breaking Abstract***. Proceedings of American Association of Cancer Research 104th Annual Meeting. ***Cancer Res.*** 73(8), Suppl. 1; doi:10.1158/1538-7445.AM2013-LB-154 154 (2013).

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5. **Garg, R**., Benavides, F., Blando, J., Perez, C.J., Abba, M., and Kazanietz, M.G. PKCε cooperates with Pten loss for prostate cancer progression. Manuscript submitted (under review).

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APPENDICES

FIGURE LEGENDS:

Figure 1: Enhanced signaling alterations in lesions from PKCε transgenic mice. (A) Immunohistochemical analyses for Akt, phospho-Akt, S6, phosphor-S6, Stat3, phospho-Stat3 staining was performed on ventral prostates from PB-PKCε, Pten, PB-PKCε;Pten or wild-type FVB/N male mice at 12 months. (B) Immunohistochemical analyses for phospho-NF-κB staining was performed on ventral prostates from PB-PKCε, Pten, PB-PKCε;Pten or wild-type FVB/N male mice at 12 months. (C) Protein expression of Akt, phospho-Akt, mTOR, phospho-mTOR, (D) Stat3 and phospho-Stat3 in total cell extracts from prostates of mice (12 months of age) belonging to different groups was analyzed by western blot. (E) Protein expression of

NF-κB in nuclear extracts from prostates of mice (12 months of age) belonging to different groups was analyzed by western blot.

Figure 2: Enhanced COX-2 activation in lesions from PKCε;Pten transgenic mice. Protein expression of COX-2 in total cell extracts from prostates of mice (12 months of age) belonging to different groups was analyzed by western blot.

Figure 3: COX-2 inhibition impairs the activation of signaling pathways in adenocarcinomas formed as a consequence of PKCε overexpression and Pten loss. PB-PKCε;Pten mice were divided in 2 groups. Group 1 received the control laboratory diet. Group 2 mice were fed with rofecoxib diet. Protein expression of (A) Akt, phospho-Akt, mTOR, phospho-mTOR, (B) Stat3 and phospho-Stat3 in total cell extracts from prostates of mice (12 months of age) belonging to different groups was analyzed by western blot.

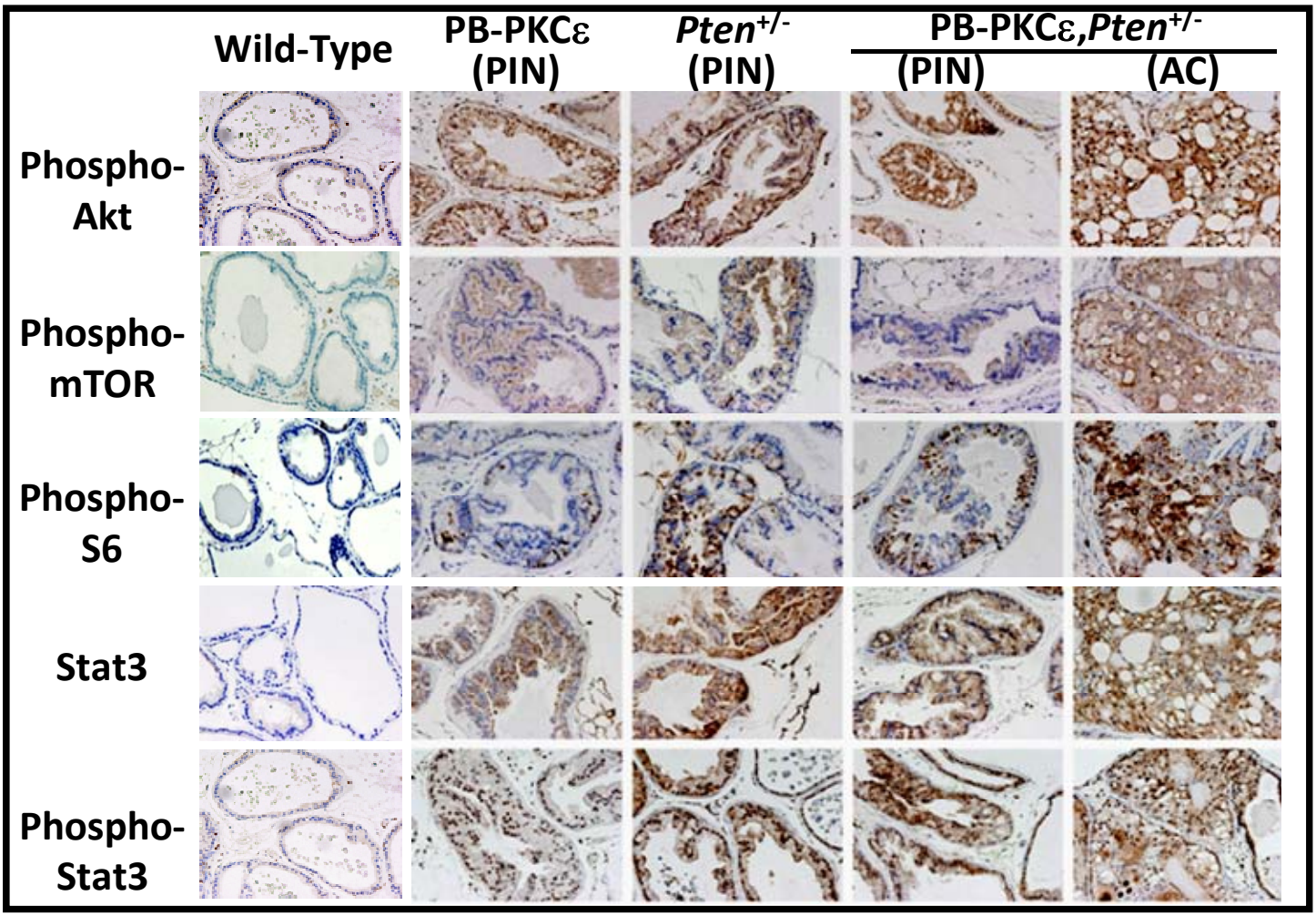
Figure 4: PKCε overexpression and Pten loss cooperate for Erk, Akt and mTOR activation. Murine prostate epithelial cells were serum-starved for 48 h and then stimulated with PDGF (5 ng/ml) for 2 min. Effect of PKCε overexpression and Pten loss were analyzed on the activation of Akt, mTOR and Erk signaling pathway by western blot analysis. Representative blots are shown.

Figure 5: Gene expression analysis in murine prostate epithelial cell lines. (A) Venn diagrams representing the regulated genes in different genotypes (+/- 2-fold; q<0.05). (B) Heat Map. (C) Enriched gene-ontology (GO) processes and biological pathways in different backgrounds owing to PKCε overexpression and Pten loss with respect to P8 as control. (D) Heat map showing genes deregulated in human prostate cancer (GSE6919 database). Changes in CaP8-PKCε cells display a similar pattern of expression as observed in human prostate cancer metastasis.

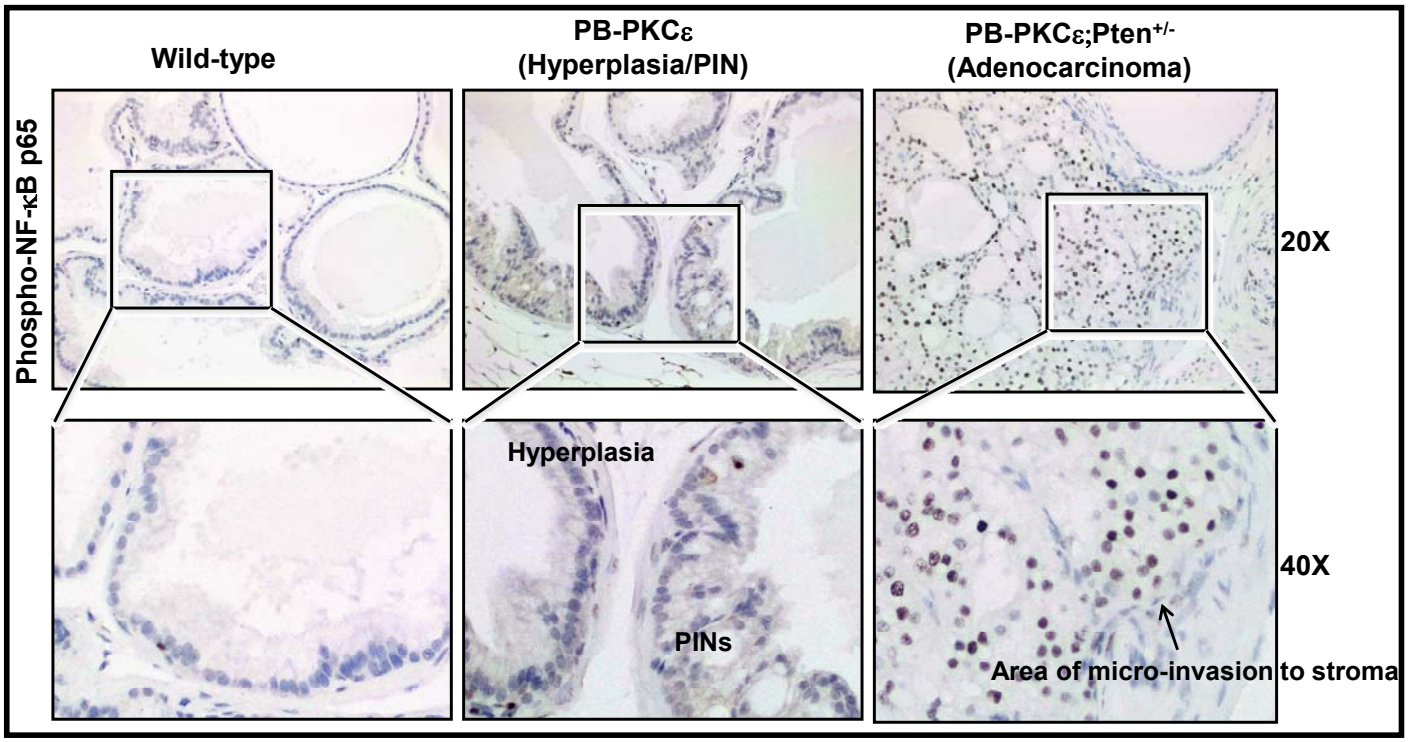
Figure 6: Inhibition of both canonical and non-canonical NF-κB pathway decreased the COX-2 activation mediated by PKCε overexpression and Pten loss. (A) Cells were serum-starved for 48 h and then stimulated with LPS 5 μg/ml for 4h. Effect of PKCε overexpression and Pten loss were analyzed on COX-2 mRNA expression by qPCR. *, p<0.05 vs control; n=3. (B) CaP8-PKCε cells were transfected with non-target control (*NTC)* or siRNA duplexes of NIK, IKKα, IKKβ or (C) with plasmids encoding IκBα super-repressor (IκBα^r) or vector control. Cells were serum-starved and subsequently stimulated with LPS (5 μg/ml, 4 h). Effect of inhibition of canonical or non-canonical NF-kB signaling was analyzed on the expression of NF-kB-dependent genes: *COX-2* by qPCR. (D) Western blot analysis was done to confirm the depletion by different siRNA dulplexes, and NF-κB luciferase activity was measured to validate repression by IkBa^r . *, p<0.05 *vs* control.

Figure 1

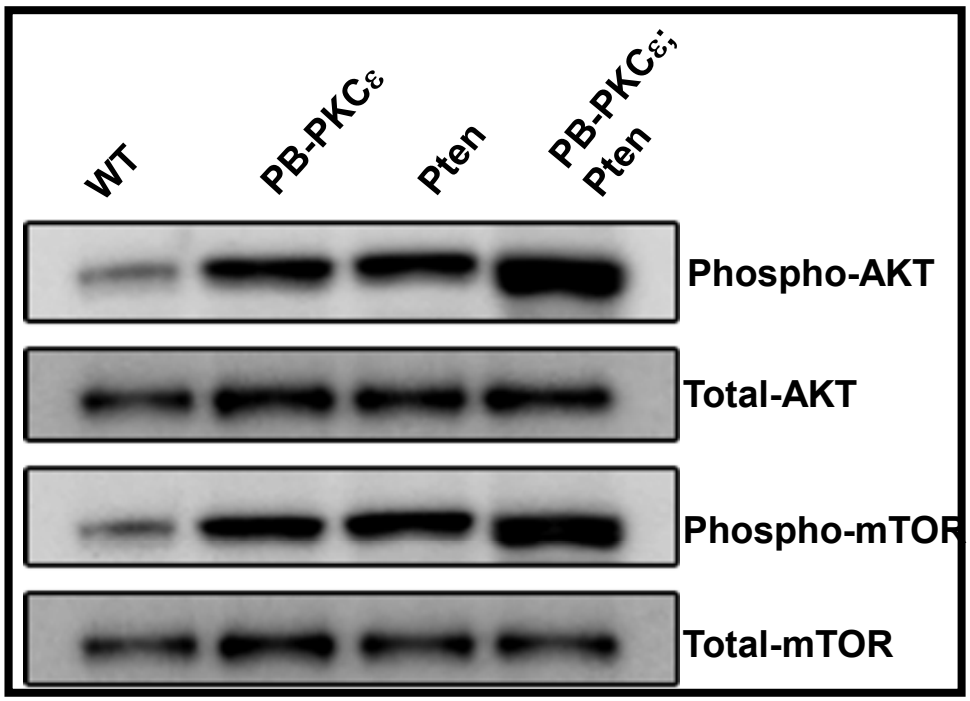
A


Wild-Type
PB-PKCε (PIN)
Pten+/- (PIN)
PB-PKCε,Pten+/-
(PIN)
(AC)
Phospho-Akt
Phospho-mTOR
Phospho-S6
Stat3
Phospho-Stat3

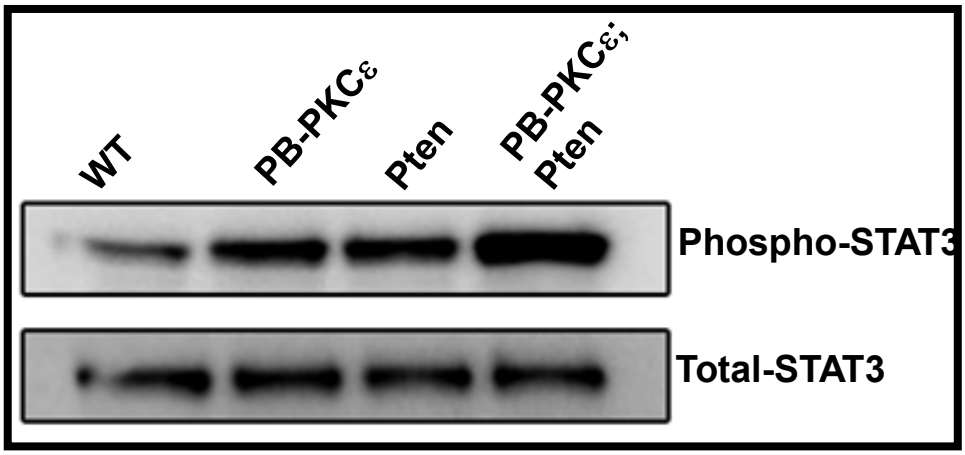
B


Wild-type
PB-PKCε
(Hyperplasia/PIN)
PB-PKCε;Pten+/-
(Adenocarcinoma)
Phospho-NF-κB p65
20X
Hyperplasia
PINs
40X
Area of micro-invasion to stroma

C


WT
PB-PKCε
Pten
PB-PKCε; Pten
Phospho-AKT
Total-AKT
Phospho-mTOR
Total-mTOR

D


WT
PB-PKCε
Pten
PB-PKCε; Pten
Phospho-STAT3
Total-STAT3

E

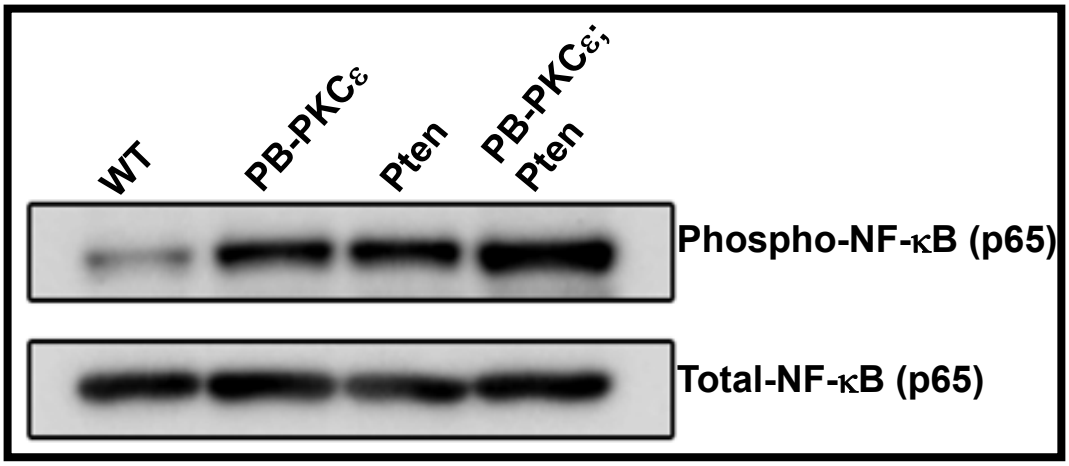

WT
PB-PKCε
Pten
PB-PKCε; Pten
Phospho-NF-κB (p65)
Total-NF-κB (p65)

Figure 2

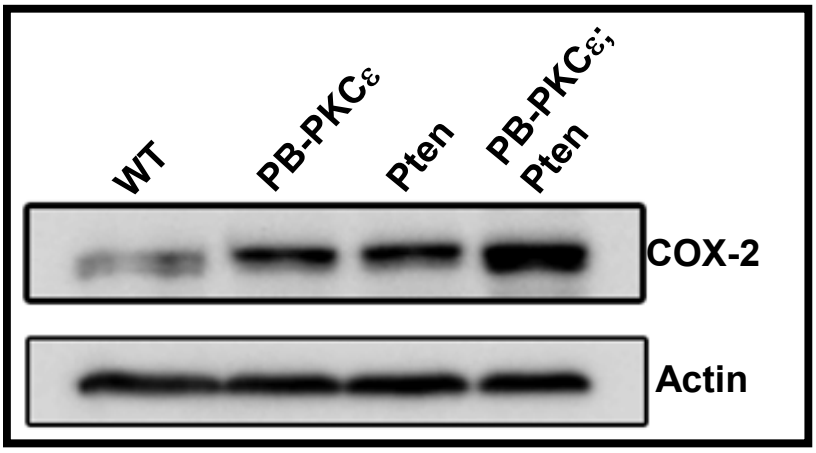
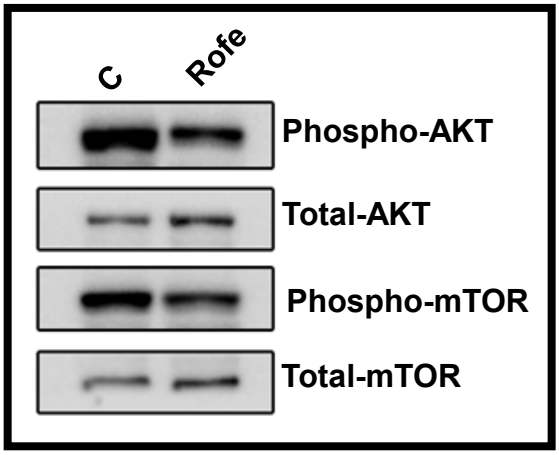


Figure 3

A



B

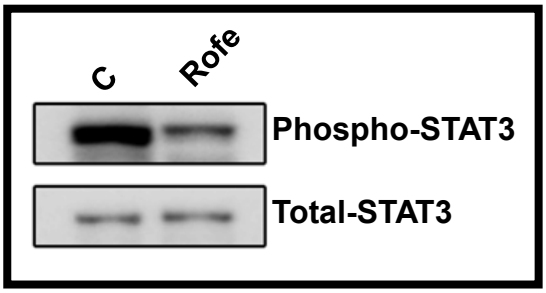


Figure 4

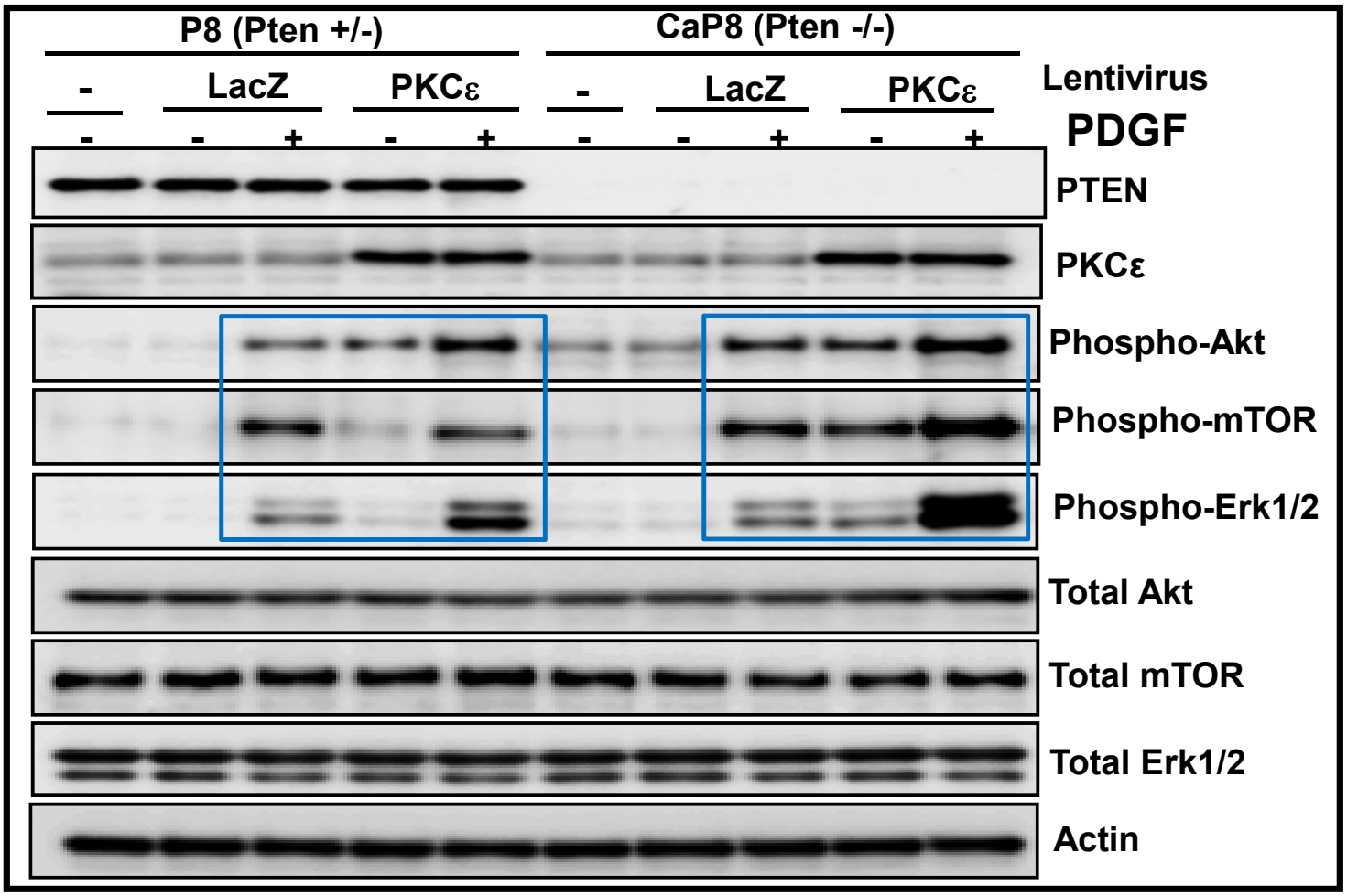
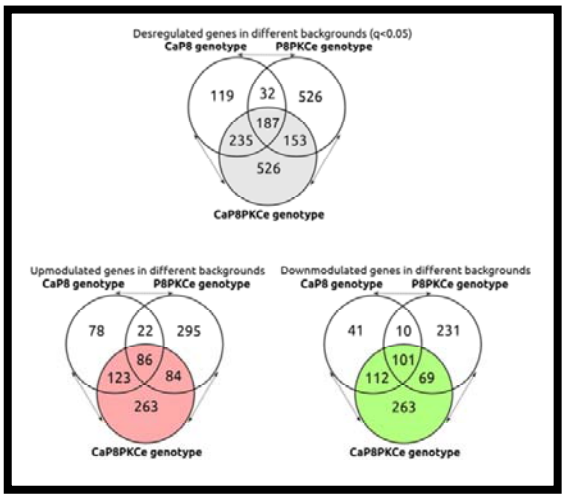
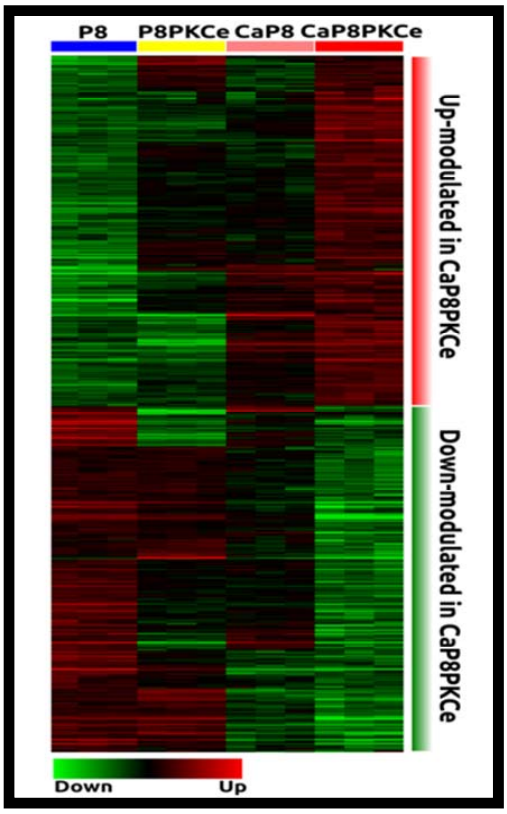

P8 (Pten +/-)
CaP8 (Pten -/-)
-
LacZ
PKCε
-
LacZ
PKCε
Lentivirus
- - + - + - - + - +
PDGF
PTEN
PKCε
Phospho-Akt
Phospho-mTOR
Phospho-Erk1/2
Total Akt
Total mTOR
Total Erk1/2
Actin

Figure 5

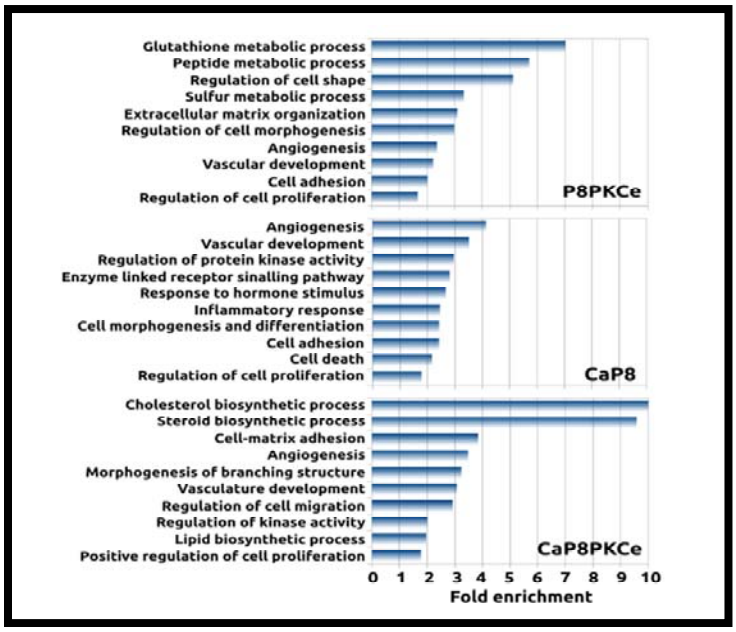
A


Desregulated genes in different backgrounds (q<0.05)
CaP8 genotype
P8PKCe genotype
119
32
526
187
235
153
526
CaP8PKCe genotype
Upmodulated genes in different backgrounds
CaP8 genotype
P8PKCe genotype
78
22
295
86
123
84
263
CaP8PKCe genotype
Downmodulated genes in different backgrounds
CaP8 genotype
P8PKCe genotype
41
10
231
101
112
69
263
CaP8PKCe genotype

B


P8
P8PKCe
CaP8
CaP8PKCe
Up-modulated in CaP8PKCe
Down-modulated in CaP8PKCe
Down
Up

C


Glutathione metabolic process
Peptide metabolic process
Regulation of cell shape
Sulfur metabolic process
Extracellular matrix organization
Regulation of cell morphogenesis
Angiogenesis
Vascular development
Cell adhesion
Regulation of cell proliferation
P8PKCe
Angiogenesis
Vascular development
Regulation of protein kinase activity
Enzyme linked receptor sinalling pathway
Response to hormone stimulus
Inflammatory response
Cell morphogenesis and differentiation
Cell adhesion
Cell death
Regulation of cell proliferation
CaP8
Cholesterol biosynthetic process
Steroid biosynthetic process
Cell-matrix adhesion
Angiogenesis
Morphogenesis of branching structure
Vasculature development
Regulation of cell migration
Regulation of kinase activity
Lipid biosynthetic process
Positive regulation of cell proliferation
CaP8PKCe
0 1 2 3 4 5 6 7 8 9 10
Fold enrichment

D

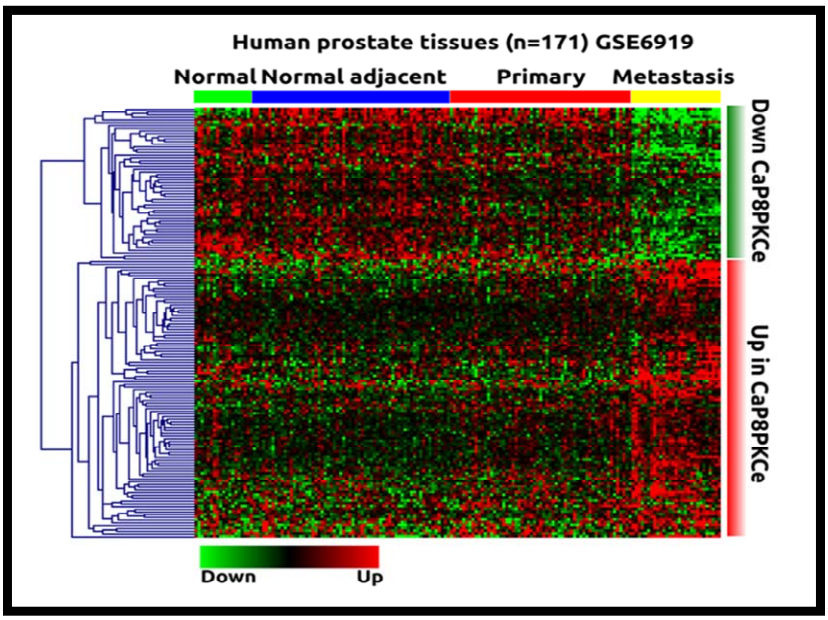
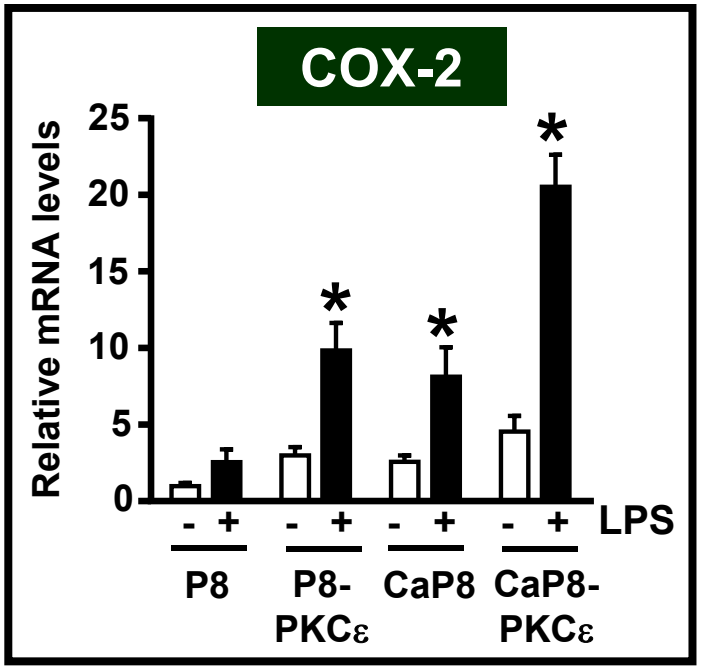
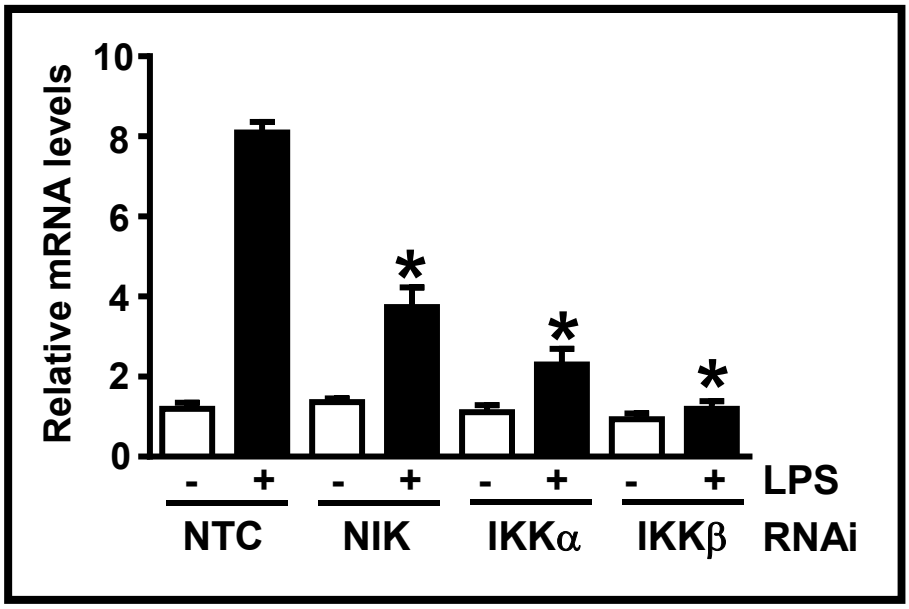

Human prostate tissues (n=171) GSE6919
Normal
Normal adjacent
Primary
Metastasis
Down CaP8PKCe
Up in CaP8PKCe
Down
Up

Figure 6

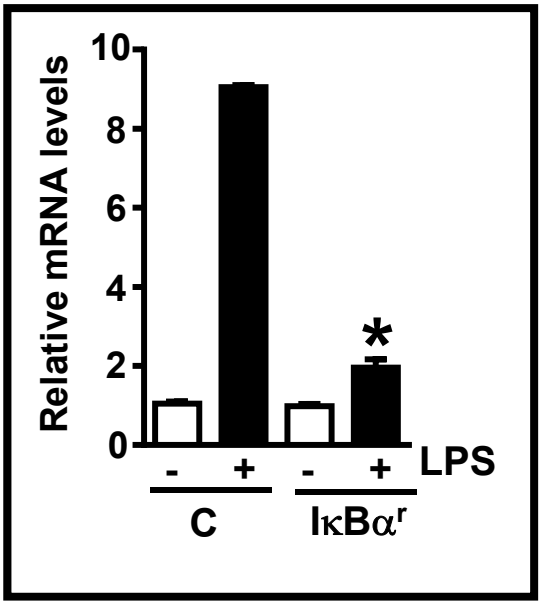
A


COX-2
Relative mRNA levels
25
20
15
10
5
0
*
*
*
- + - + - + - +
LPS
P8
P8-PKCε
CaP8
CaP8-PKCε

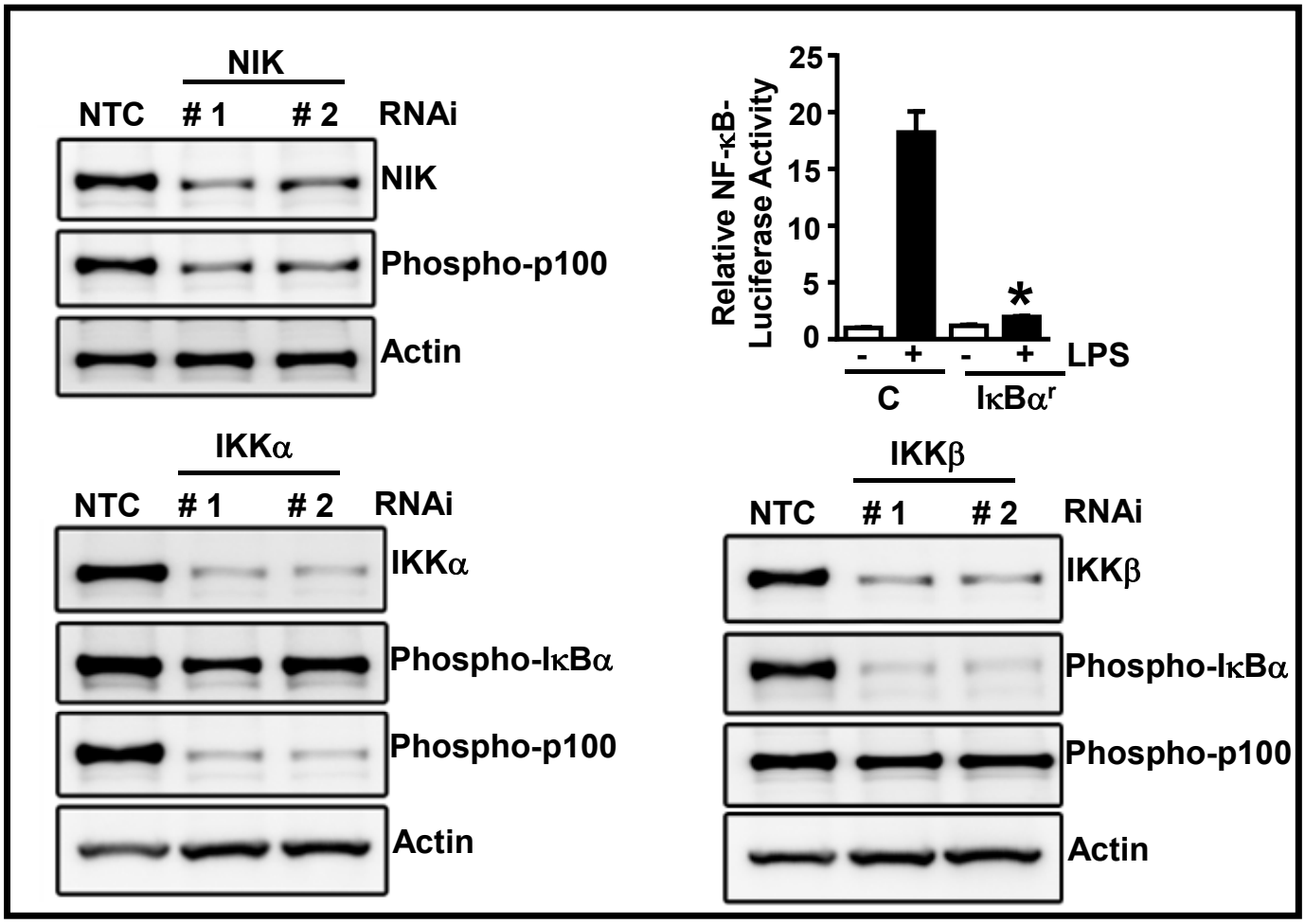
B


Relative mRNA levels
10
8
6
4
2
0
*
*
*
- + - + - + - +
LPS
NTC
NIK
IKKα
IKKβ
RNAi

C


Relative mRNA levels
10
8
6
4
2
0
*
- + - +
LPS
C
IκBα^r

D


NIK
NTC
1
2
RNAi
NIK
Phospho-p100
Actin
Relative NF-κB-Luciferase Activity
25
20
15
10
5
0
*
- + - +
LPS
C
IκBα^r
IKKα
NTC
1
2
RNAi
IKKα
Phospho-IκBα
Phospho-p100
Actin
IKKβ
NTC
1
2
RNAi
IKKβ
Phospho-IκBα
Phospho-p100
Actin